Microsatellite diagnosis of species within the *Ceratitis fasciventris, C. anonae, C. rosa* species complex (Diptera: Tephritidae)

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2nd CRP Meeting Cryptic Species, Brisbane 2012

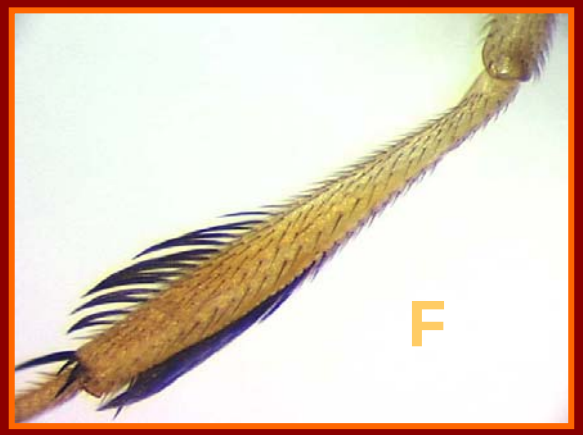
Current taxonomy

Three recognized species:

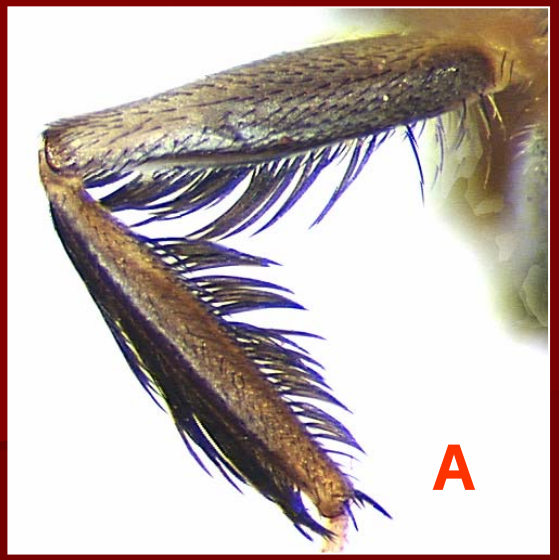
- *Ceratitis fasciventris*
- *Ceratitis anonae*
- *Ceratitis rosa*

Male differentiation

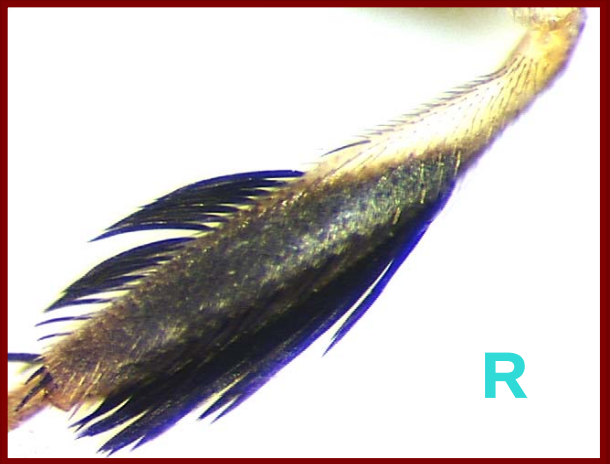
Based on secondary sexual characters: leg feathering



C. fasciventris



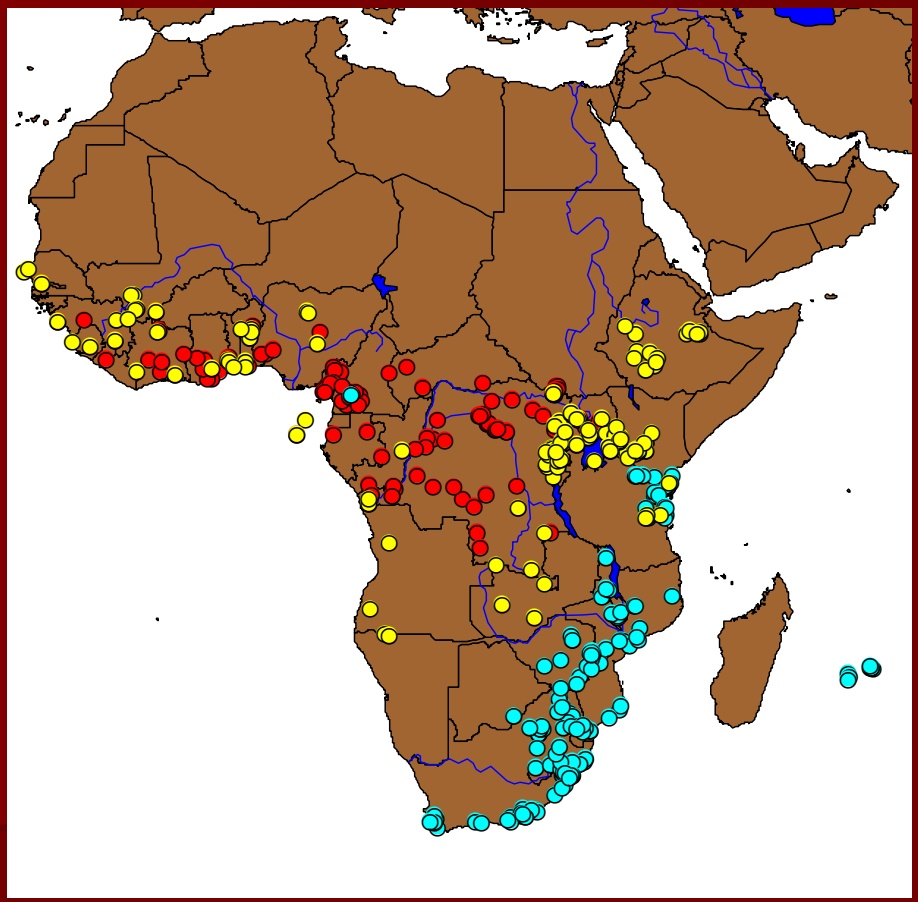
C. anonae



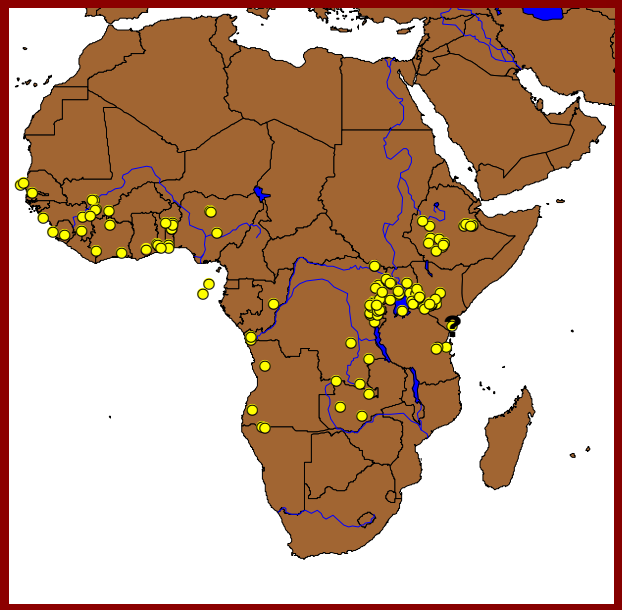
C. rosa

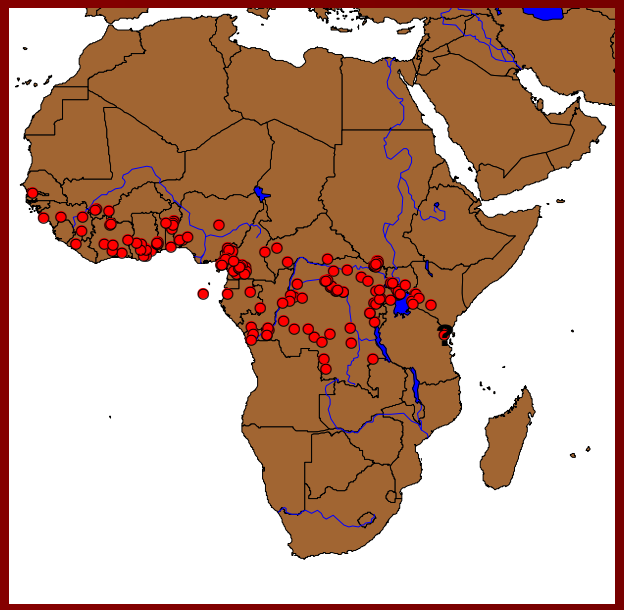
Distribution

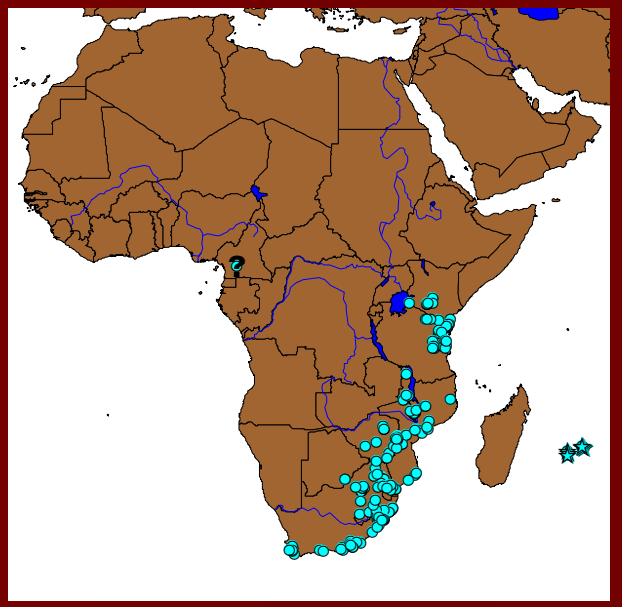
Complex widespread through Africa



Different distribution patterns







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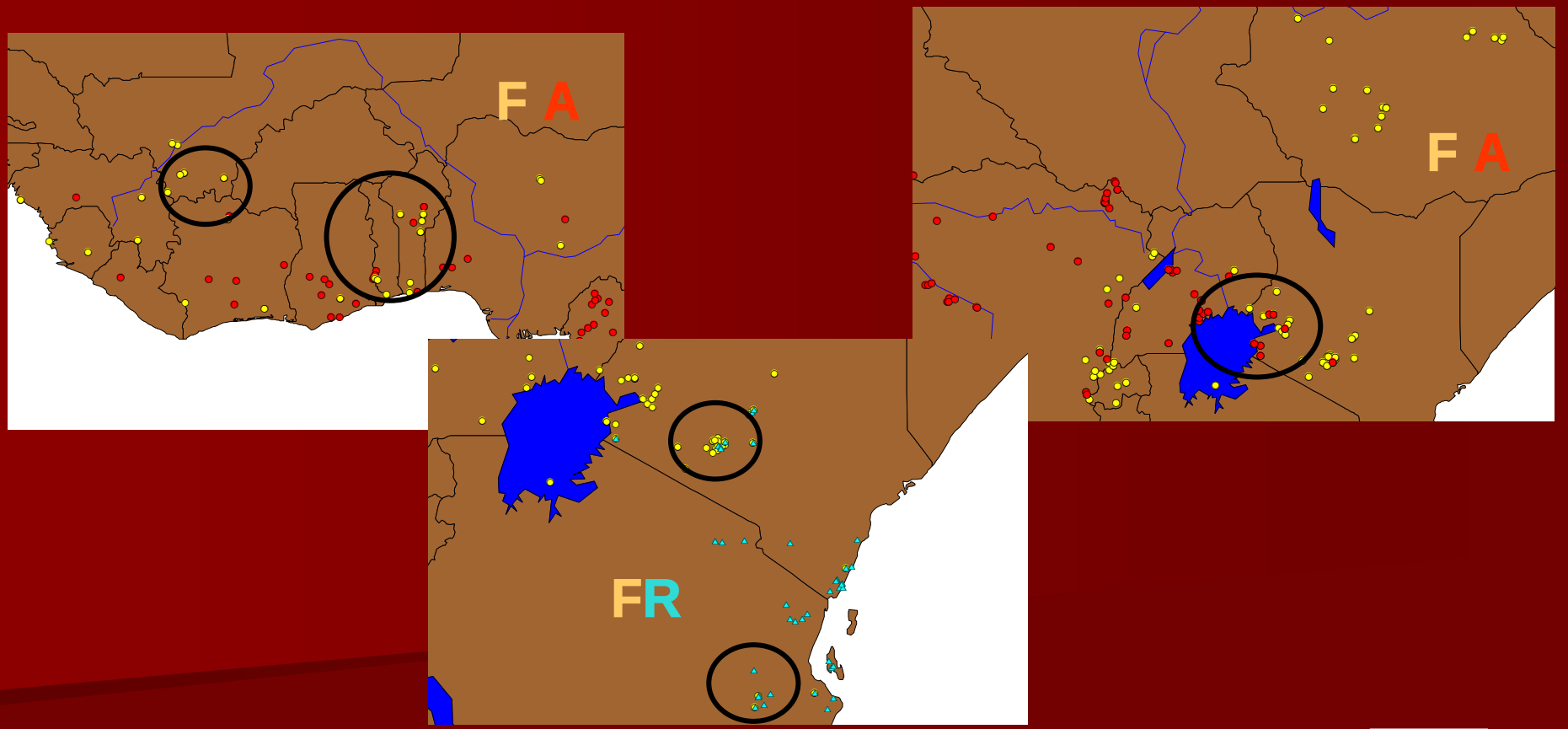
Sympatric or parapatric occurrence

Areas where two species co-occur



Intra-specific variation

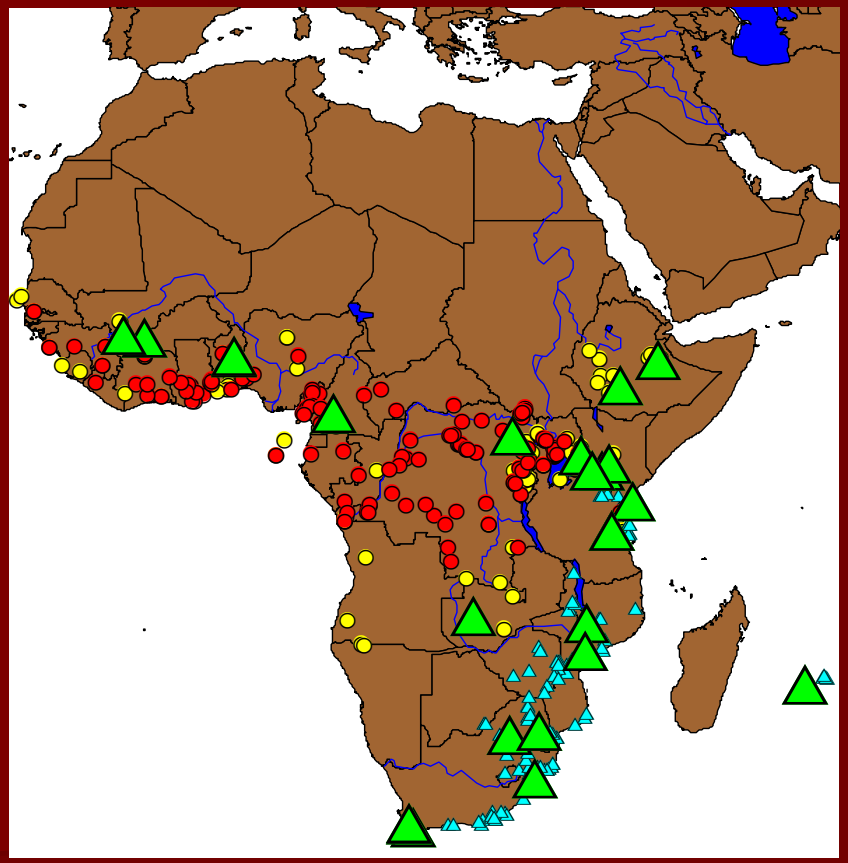
Leg coloration male *C. fasciventris*

West versus East African populations

Sample selection

27 samples
621 individuals
12 African countries

C. rosa	12 samples, n: 296
C. fasciventris	10 samples, n: 214
C. anonae	5 samples, n: 111



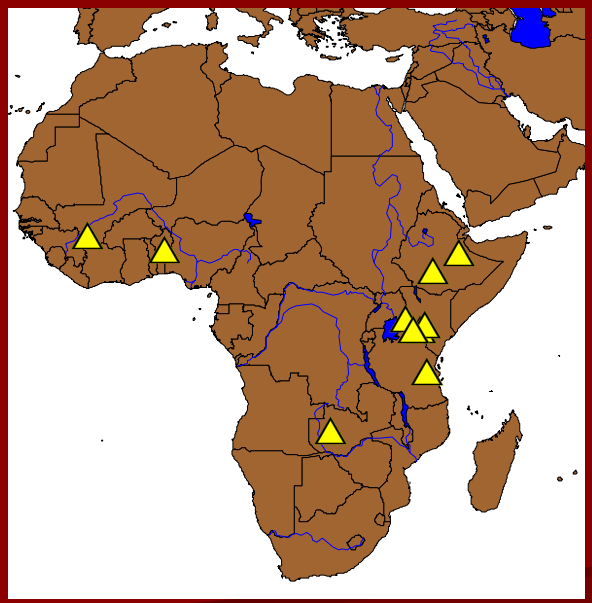
Sample selection

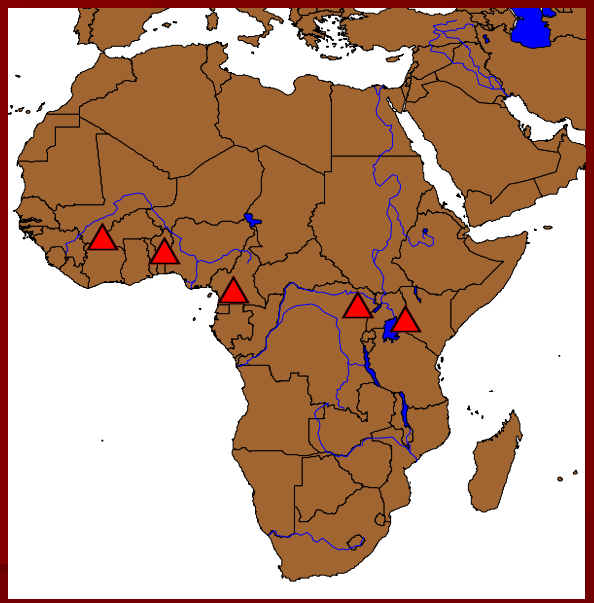
10F/5A/12R
4 sympatric or parapatric occurrences

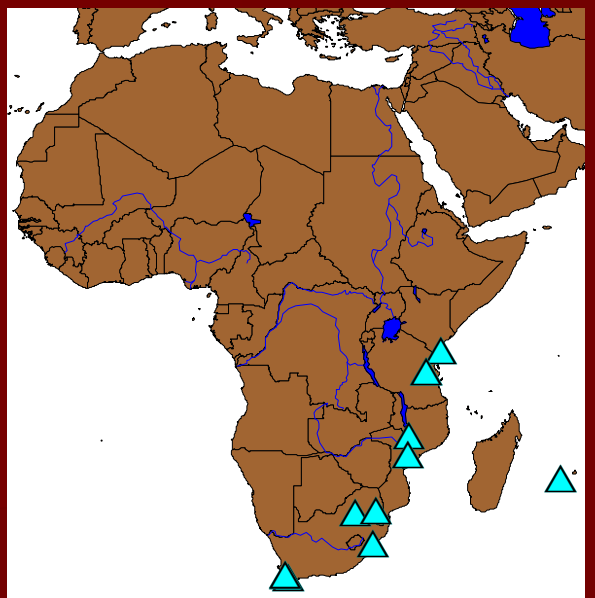
sympatric *C. fasciventris* - *C. anonae*
(Kenya - Kakamega forest)

parapatric *C. fasciventris* - *C. anonae*
(Benin – Komin, Benin –Parakon)

sympatric *C. fasciventris* - *C. rosa*
(Tanzania - Morogoro/SUA)







Africa Africa
TERVUREN

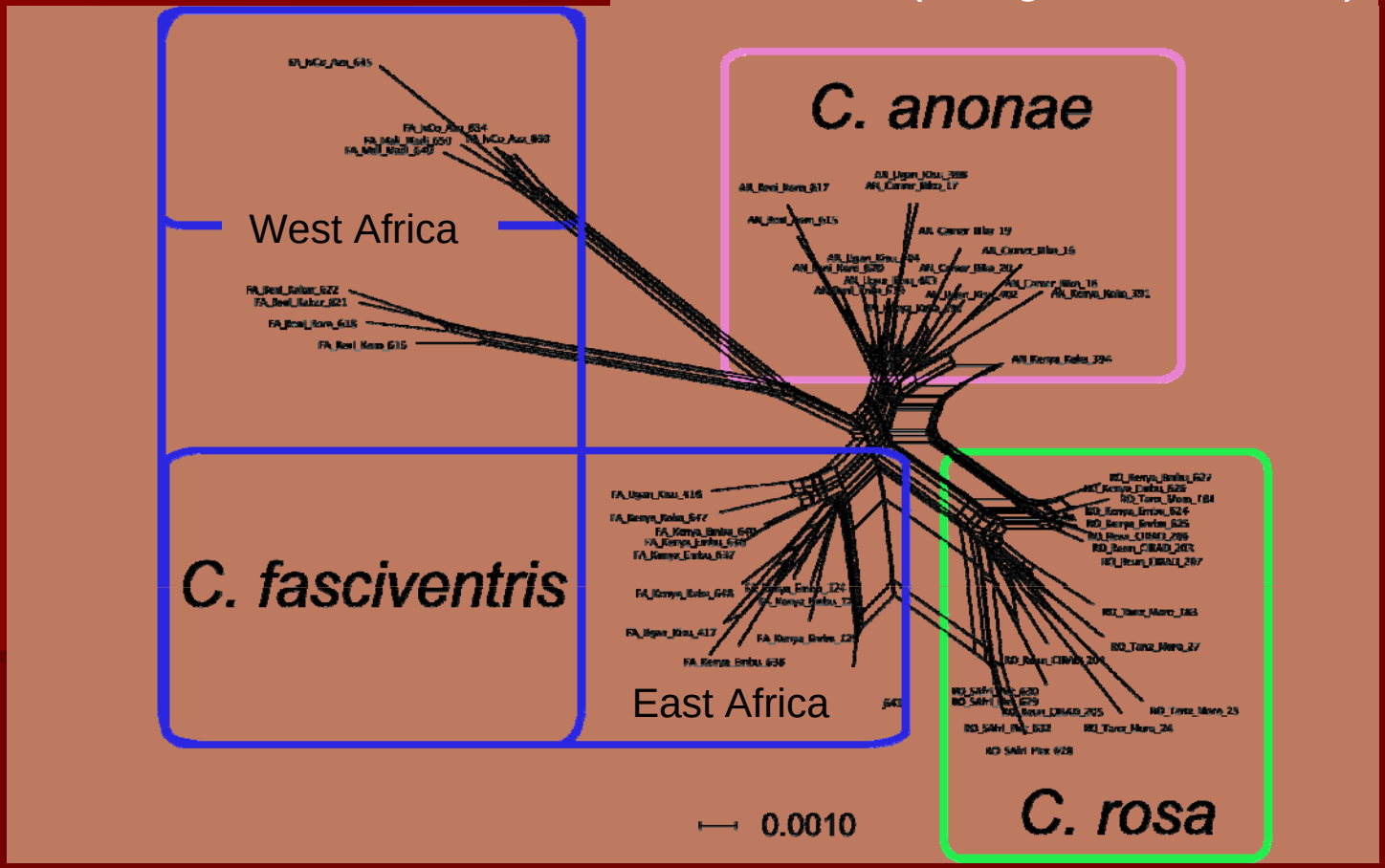
sequence analysis: lack of resolution...

Virgilio, Backeljau, Barr, De Meyer (2008) *Molecular Phylogenetics and Evolution 48, 270-280*

concatenated Per – 16S – COI – ND6

2657 base pairs

NJT network (K2P genetic distance)

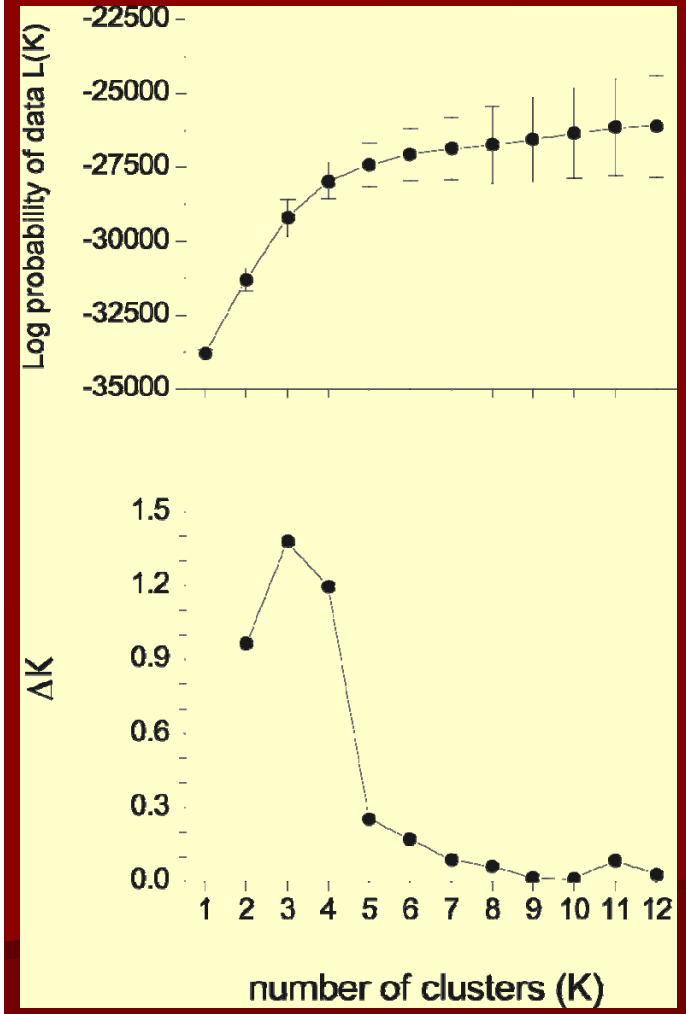


microsatellites

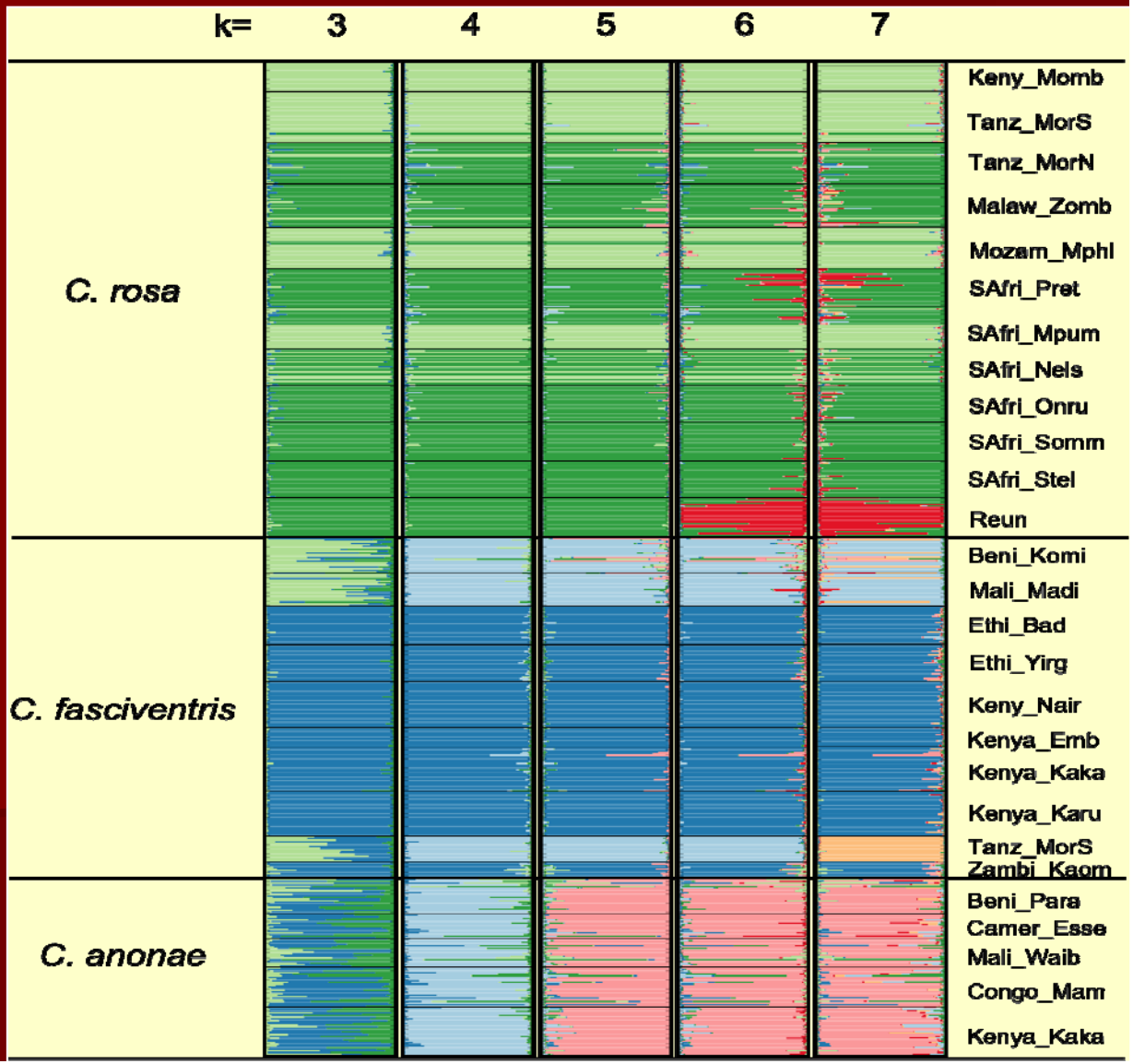
16 loci developed and tested in collaboration with CIRAD – La Réunion

locus	F pimer	R pimer	dye	repeat	length range
F_1	AGGTTGTGGTATCTACGAAAGG	GCCTGCAAATCTTTAGCAACA	6FAM	caa	70-110
F_2	GAGAAGGTAGGCAACGGATG	TTGCGTTTTGTATACGCTCG	6FAM	atc	160-200
F_3	AGGCATCACATGCACTCCTT	TGGGGAGGCAAGACAAATAA	VIC	ca	80-120
F_4	ATGAAAAAGACACCGCTTGC	AGCGGCATAACCGTAACAAT	VIC	tg	160-200
F_5	TCACAAGACGATATTTAAGTTGCT	GCACCTGCACTTGTCACACT	PET	tgt	70-100
F_6	ATATTCCCCAATTCAGTGCC	TTGCACAAGTACCAGTTCTACGA	PET	tg	250-300
F_7	AAATGCAGATAATGCAAGCG	TCAACAGCTTTGCTCTCAGTG	NED	ga	80-110
F_8	AAATCAAACACGGTTACAACCAC	ATCTAATTCTTCTTTTCGACGGC	NED	caa	230-300
F_9	TTTGAGTCTCCGAAAGCAGC	CGAAATAGAGCCAAATCGAA	6FAM	ttg	100-150
F_10	CCAGCCACCAACAGTTTTCT	TTTGTGTGGGTGGCACTTTA	6FAM	aca	195-250
F_11	TTCCTCTTACACCACCAGCC	CGTCTGTCGTCATTCCGTTA	VIC	atgt	90-120
F_12	TTATTTTACTTGCGTCCGCTT	GTATGTCCAGTACGAGGGCG	VIC	tgta	170-200
F_13	TTTACCGTTGCCACTTTTCA	AACTGCAACCAGCAGAGTCA	PET	atc	140-210
F_14	TATCTGCCTTTCCGAAGCTC	CGTCGTCGATTCTACGGACTA	PET	ac	260-290
F_15	CTTGTGGCAAGCAAGTGAAG	AAATGTGCTATAAATGGCTATGG	NED	gtat	80-130
F_16	CAAATAAATTCCTTCTTCTCTTCTTC	GTGTTGTTGCCGTTGCTTC	NED	cac	220-300

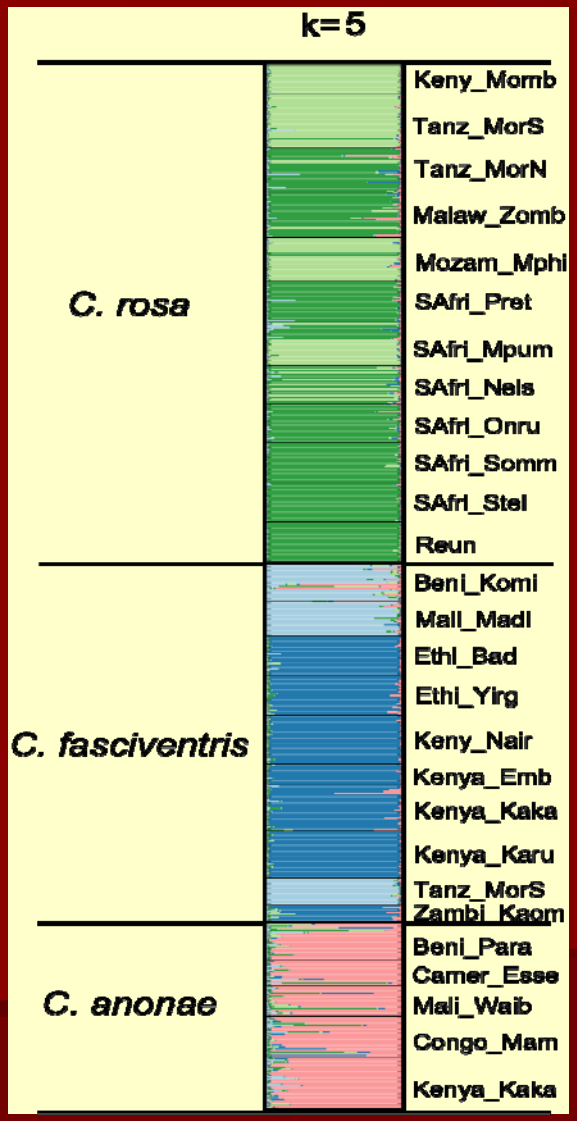
individual Bayesian assignments (STRUCTURE): how many groups?



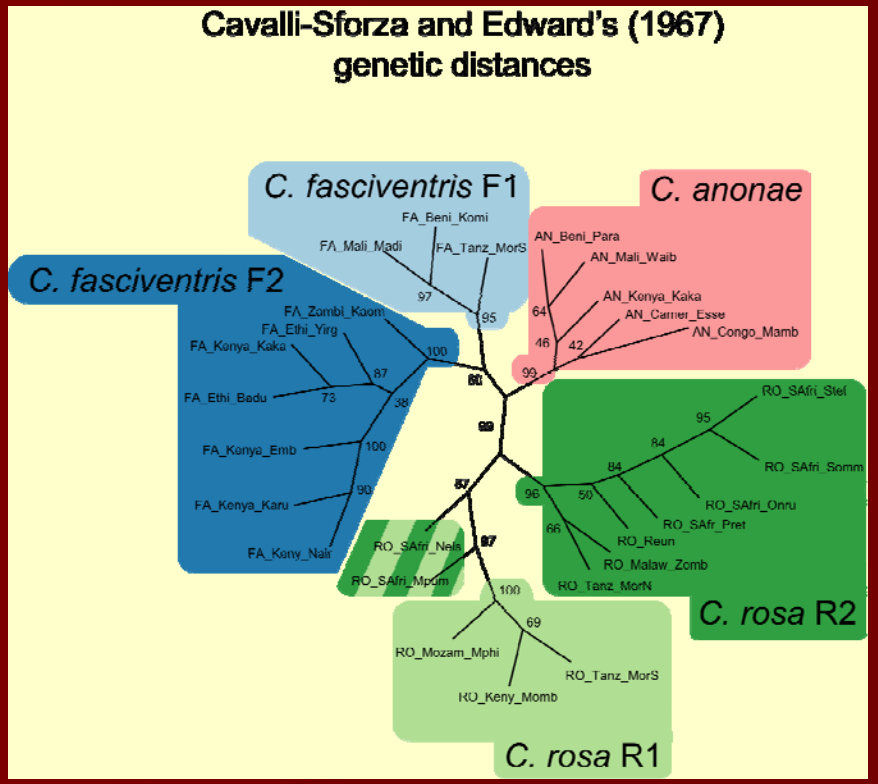
Evanno et al. (2005)



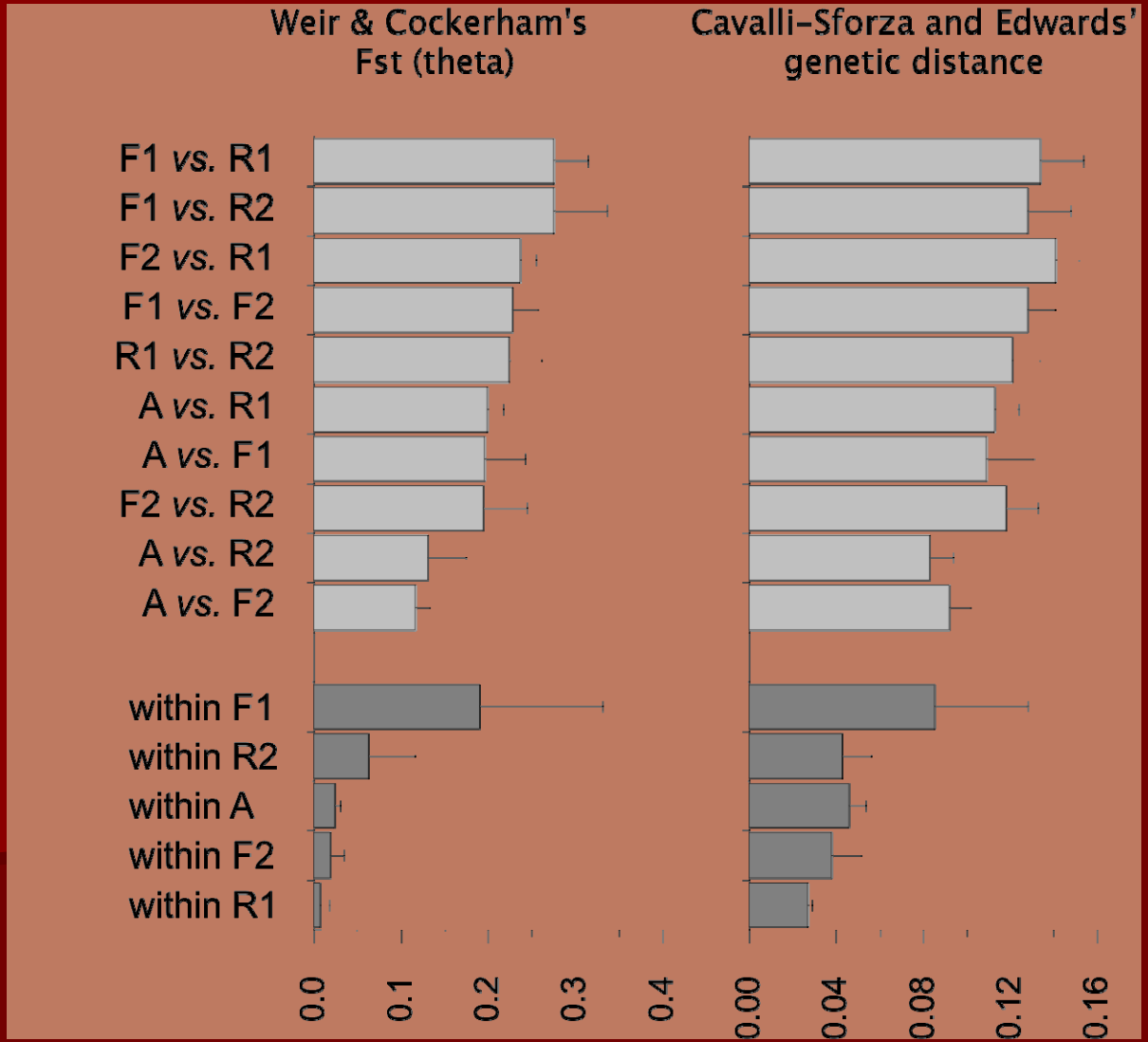
individual assignments



population distances



average genetic differentiation among types R1, R2, F1, F2, A



microsatellites diagnosis
of FAR morphospecies:

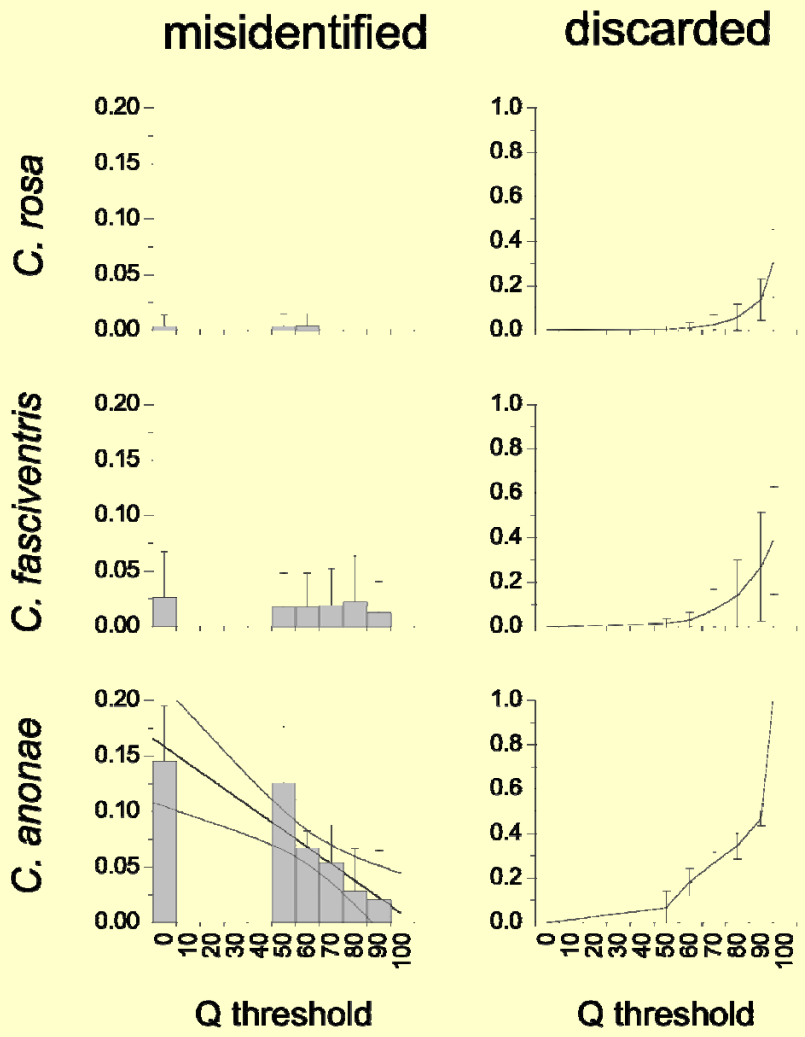
R1 or R2 -> *C. rosa*

F1 or F2 -> *C. fasciventris*

A -> *C. anonae*

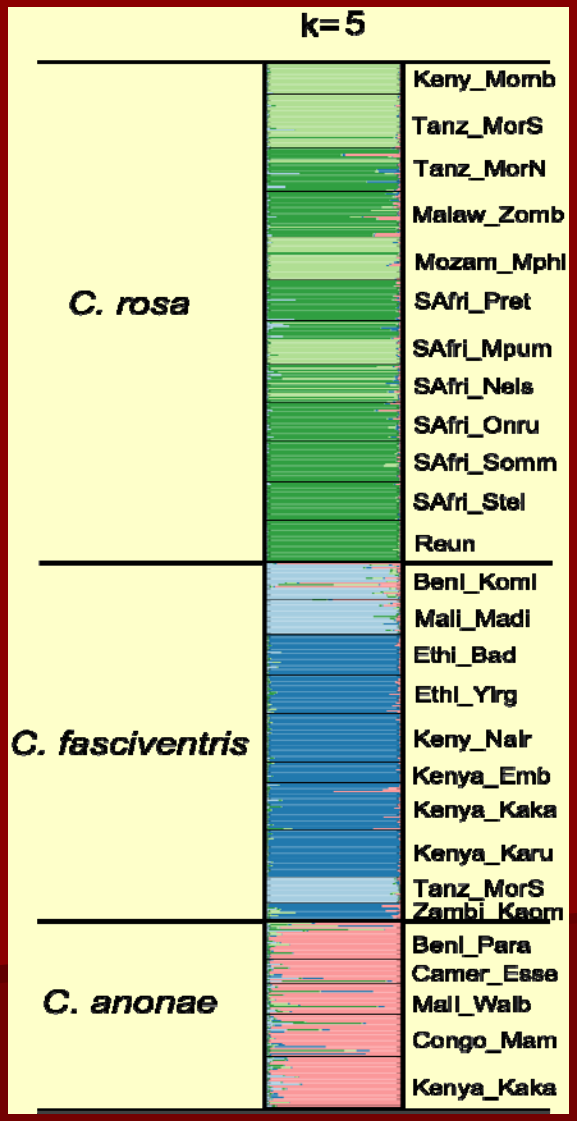
do we need an ID threshold?

molecular diagnosis FAR



proportions of misidentified and discarded specimens averaged across populations (SD as error bars)

genetic- and morpho-types



genetic types

morphotypes

males

females

R1

R2

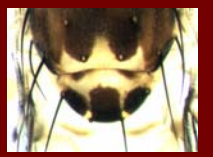
F1

F2

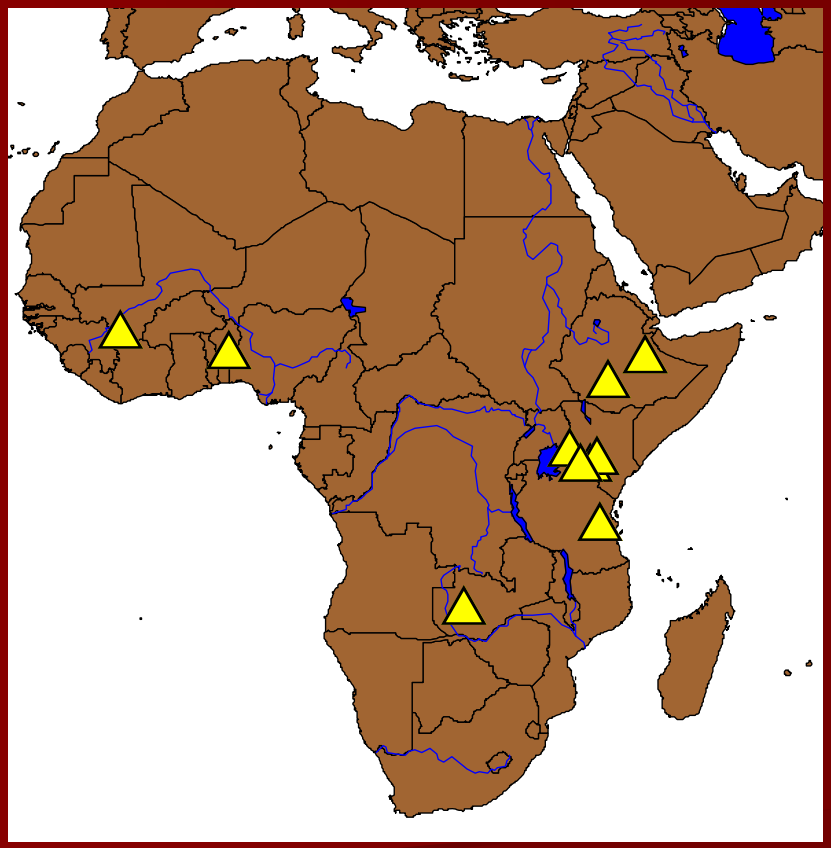
A

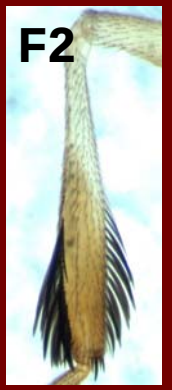
?

?



Morphotypes *C. fasciventris*





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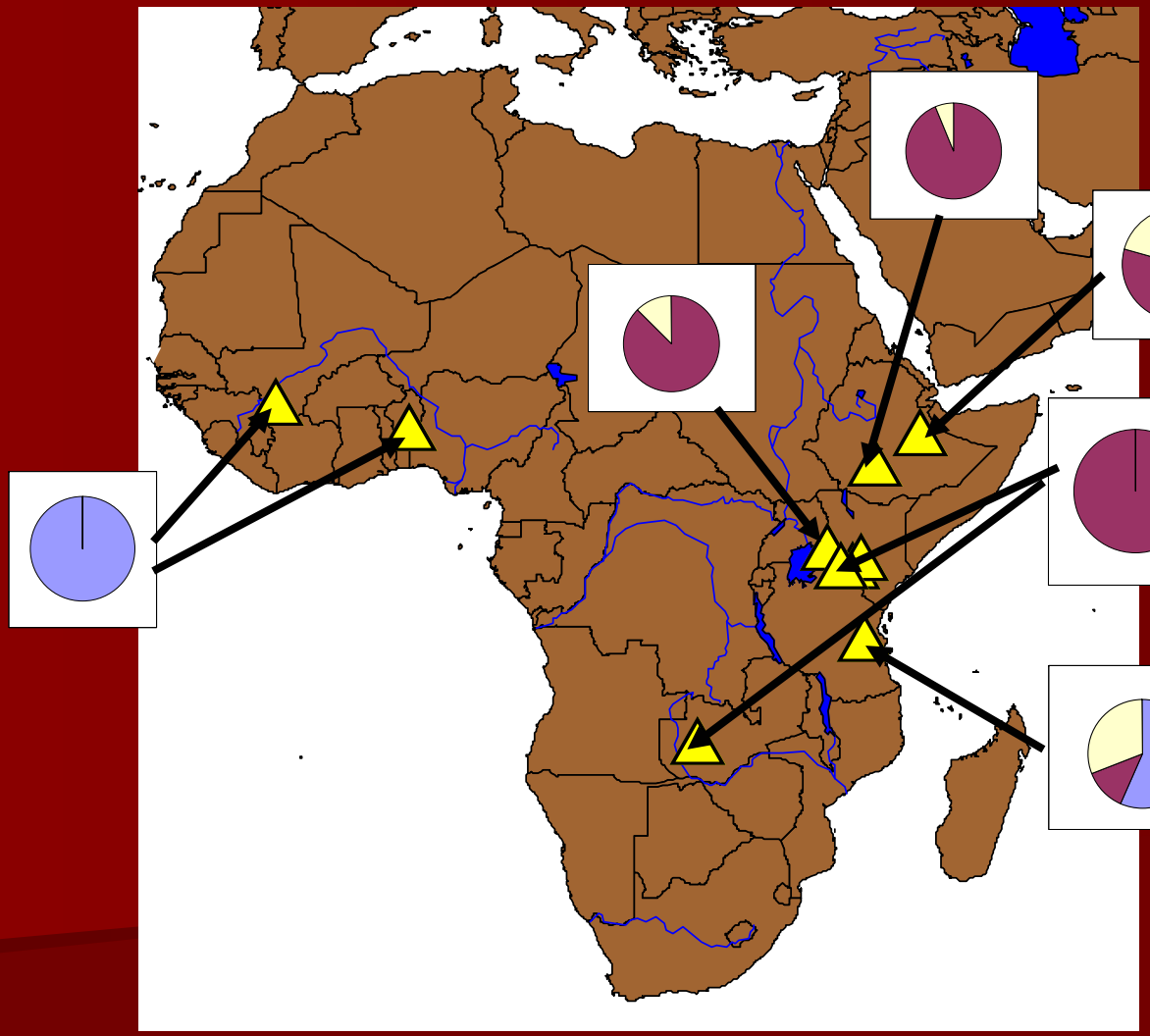
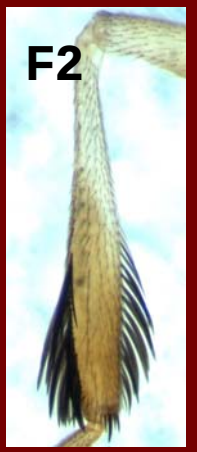
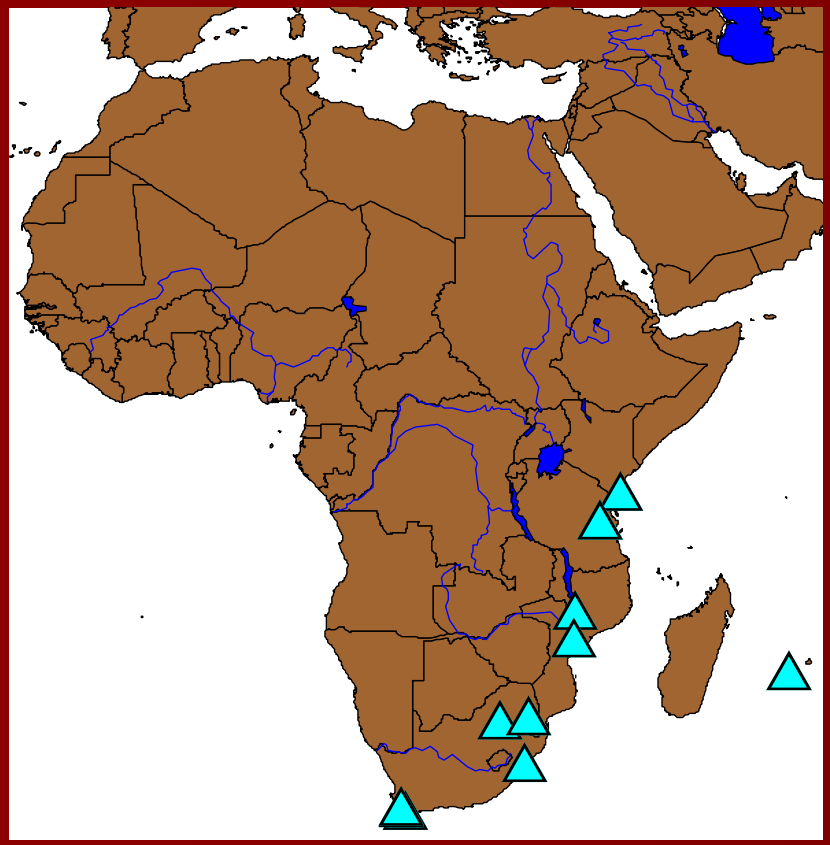
Morphotypes *C. fasciventris*

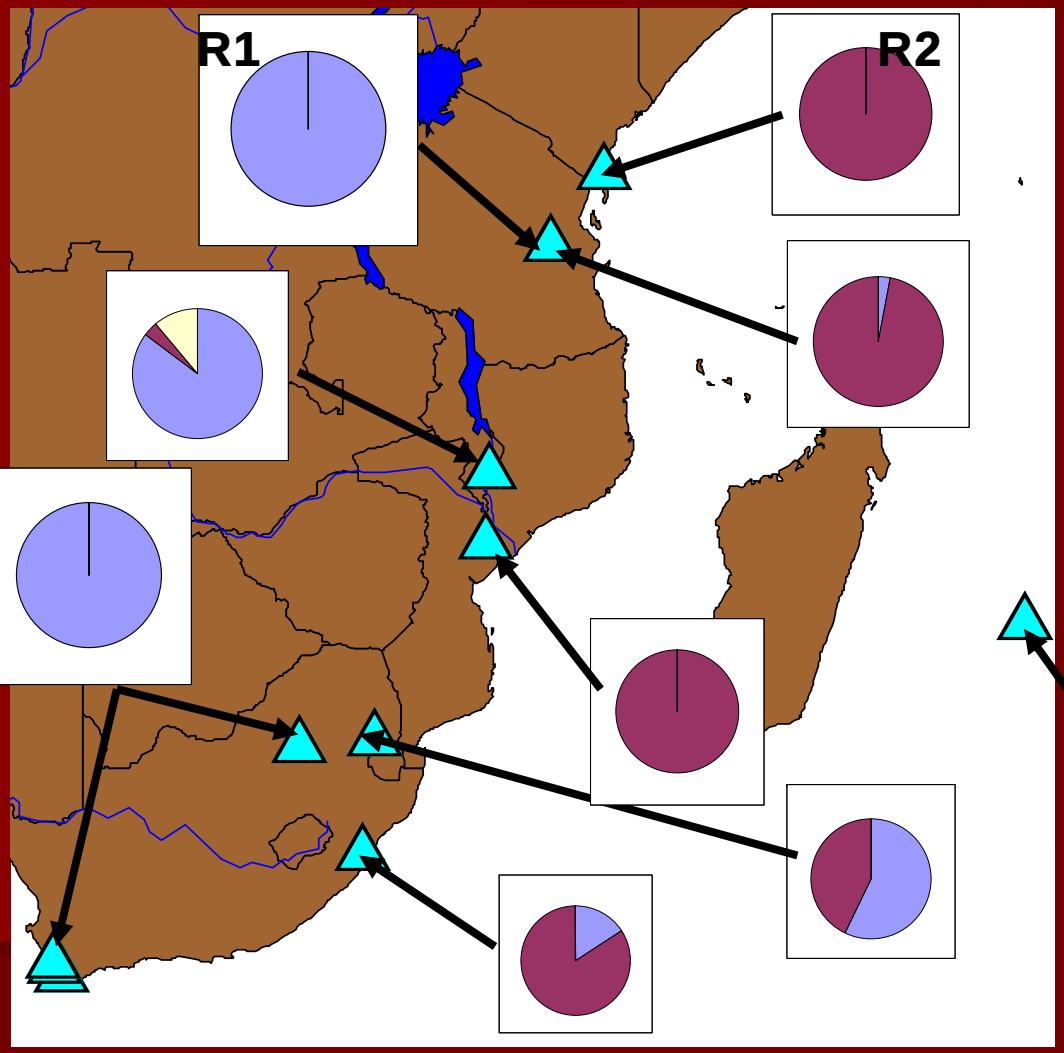




Morphotypes *C. rosa*



Morphotypes *C. rosa*



Biotypes *C. rosa* ?

Studies on developmental rates

Duyck & Quilici (2002): *C. rosa* has lower minimum temperature thresholds than C. capitata. Based on culture originating from Réunion Island

Duyck et al (2006): climatic niche partitioning with *C. rosa* preferring humid and intermediate to cold areas (wheras *C. capitata* prefers dry and intermediate to warm areas)

Grout & Stoltz (2007): *C. rosa* results differ substantially from those of Duyck et al., with higher minimum temperature threshold for development. Based on culture originating from Nelspruit supplemented with specimens from Mpumalanga Province.

Biotypes *C. rosa* ?

Réunion population corresponds to R1 type, while Nelspruit and Mpumalanga populations are partially or largely R2 type.

Tanzania: Morogoro population (400-500 masl) correspond largely to R2 type while Nyandira population (1600 masl) correspond to R1 type.

ICIPE colony corresponds to R1 type. Originates from Central Highlands. Mombasa population corresponds to R2 type.

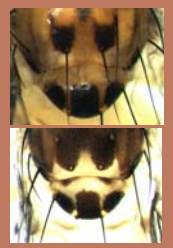
Biotypes *C. rosa* ?

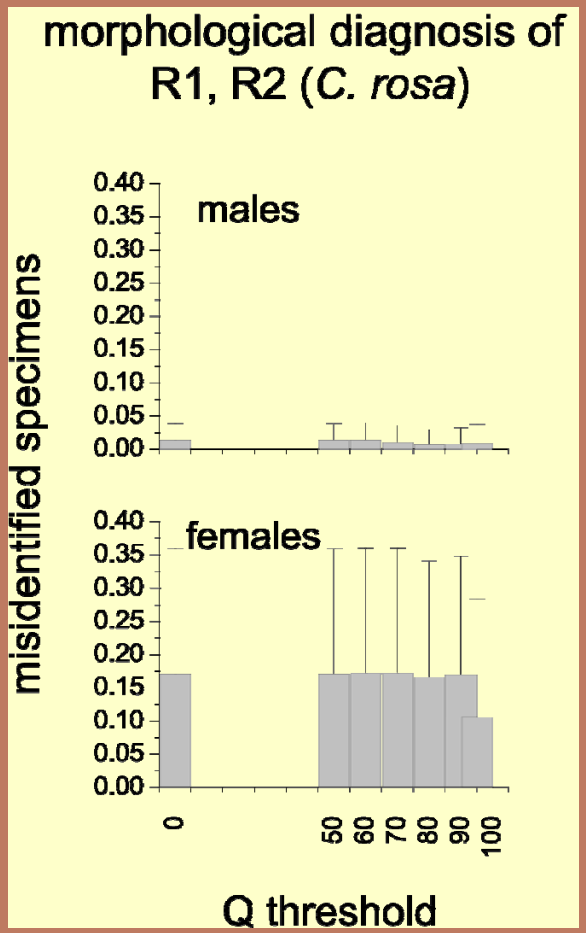
Need for further study: do the two types of *C. rosa* that are differentiated genetically and morphologically, correspond with two biotypes that have different climatic requirements regarding development?

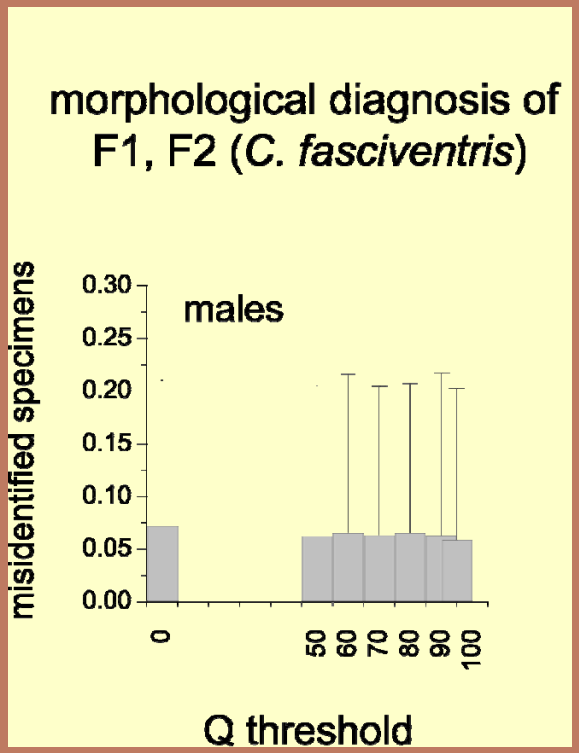
Experiment under controlled conditions should be repeated with identical conditions (same climatic chamber) and specimens studied genetically and morphologically.

If confirmed, implications for control and invasive power

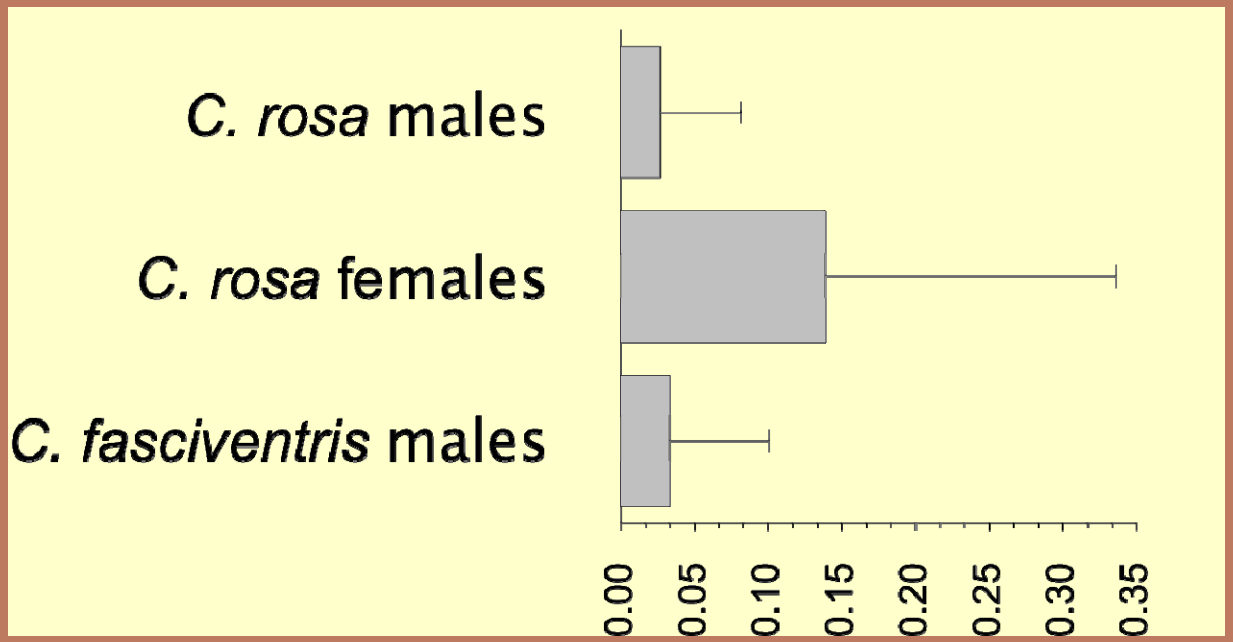
morphological diagnosis of genetic types R1, R2 (males and females) and F1, F2 (males)

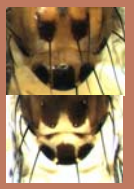






uncertain morphological diagnosis of types R1, R2, F1, F2





Summary

Micorsatelite diagnosis of FAR morphospecies (males and females)

- *C. rosa*: low ID error (<5%), highly consistent results across populations
- *C. fasciventris*: low ID error (<5%), consistent results across populations
- *C. anonae*: ID error dependent on Q threshold

Summary

Morphological diagnosis of R1, R2, F1, F2 types

- *C. rosa* males: low ID error (<5%), consistent results across populations
- *C. rosa* females: higher ID error (<10%), not consistent results across populations
- *C. fasciventris*: males ID error >15%, not consistent results across populations

proportion of morphologically-not-identified types:
C. rosa males < *C. fasciventris* males < *C. rosa* females

Future research

- Morphological characters to separate females within the FAR complex: wing landmarking
- *C. rosa* biotypes: climate chambers experiments
- Genotypes *C. fasciventris* also different biotypes?
- Differences host spectrum?
- Ecological niche modeling: data need re-analysis
- Links larval morphology, cuticular hydrocarbons

Acknowledgements

- IAEA for organizing the CRP and financial support participation of MDM
- Dick Drew & colleagues (Griffith University) for organizing this meeting
- Various collectors and collaborators in Africa for providing samples
- Action 1 Belgian Science Policy: funding research MV
- Joint Experimental Molecular Unit (Tervuren & Brussels)